Acetylation of Replication Protein A (RPA) Improves its DNA Binding Property

Sneha Surendran, Onyeka Ononye, Lata Balakrishnan.

Biology, IUPUI, Indianapolis, IN

Genome maintenance is critical for cellular survival and growth. Replication Protein A (RPA), a single-strand DNA (ssDNA) binding protein, is vital for various aspects of genome maintenance such as replication, recombination, repair and checkpoint activation. RPA binding to ssDNA protects it from degradation by cellular nucleases, prevents secondary structure formation and from illegitimate recombination. Within the cell, RPA is subject to many post-translational modifications including phosphorylation, SUMOylation and ribosylation. These modifications regulate the activity of RPA with DNA and other binding partners.

RPA has been reported to be also modified by acetylation. We found that human RPA (hRPA) can be in vitro acetylated by p300, an acetyl transferase (AT). To study the effect of this modification on its ssDNA binding function, we made use of electro-mobility gel shift assay (EMSA) and bio-layer interferometry (BLI) technology. Using various length oligos, we tested the binding property of unmodified and acetylated RPA. Our results showed that acetylation of RPA increased its binding affinity compared to unmodified RPA. Interestingly, the acetylated form was also able to bind more stably to shorter length oligos compared to the unmodified form. This suggests that the acetylation of RPA improves its ssDNA binding function. This alteration in its enzymatic activity would have significant implications in maintenance of genome fidelity since improved DNA binding function of RPA will protect the genome from both endogenous and exogenous stresses. Additionally, using mass spectrometry analysis we have identified the lysine residues that get modified by the acetyl group both in vitro and in vivo. We are currently studying the factors that trigger this post-translational modification in the cell.